Kidney International, Vol. 31 (1987), pp. 886–897

LABORATORY INVESTIGATION

Lysosomes of the renal cortex: Heterogeneity and role in protein handling

KNUT-JAN ANDERSEN, HANS-JACOB HAGA, and MILOSLAV DOBROTA

Section for Clinical Research and Molecular Medicine, Medical Department A, University of Bergen, N-5016, Haukeland Sykehus, Bergen, Norway, and Robens Institute of Industrial Environmental Health and Safety, University of Surrey, Guildford GU2 5XH, United Kingdom

Lysosomes of the renal cortex: heterogeneity and role in protein handling. Rate sedimentation of the kidney cortical mitochondrial/lysosomal (ML) fraction yields two distinct classes of lysosomes: the large lysosomes or protein droplets and a heterogenous broad band of smaller lysosomes. The protein droplets which are recovered as a well defined zone of high purity also sediment as a homogeneous band after equilibrium banding at a density of 1.235 g/ml in sucrose. The small lysosomes co-sediment with other subcellular organelles as a broad band, indicated by the distribution of various acid hydrolases, which exhibit subtle heterogeneity among these small lysosomes. The distribution of renin containing granules indicates that in size they represent a distinct subpopulation of small lysosomes. Further fractionation of small lysosomes by equilibrium banding separates two distinct populations at densities 1.20 (small light) and 1.235 g/ml (small dense). Comparison of lysosomal populations fractionated in these studies with the distribution of lysosomal acid hydrolases along the different segments of the nephron suggests that large and small dense lysosomes probably originate from the proximal tubule while the small light lysosomes may contain lysosomes from the distal tubule. Very small, lysosome-like organelles subfractionated from the 'microsomes' may constitute a mixture of small light lysosomes, lysosomal fragments and endocytic vesicles from a variety of cell types. Time course studies with ^{3}H labelled Cd-thionein, following intravenous administration, suggests that uptake in the kidney cortex is very rapid and that catabolism takes place in two distinct phases: rapid breakdown starting in the endosome compartment and slower breakdown in lysosomes. From the association of labelled lysozyme (^{125}I) and Cd-thionein (^{109}Cd) it appears that all the different lysosomal populations identified are at some stage involved with uptake and catabolism of these two proteins.

The complex cellular heterogeneity of the kidney [1] and the presence of lysosomal acid hydrolases, in varying proportions, in all the segments of the nephron [2] suggest considerable heterogeneity of lysosomal populations. This heterogeneity has indeed been observed by morphology, with kidney lysosomes varying in size from 0.1 to 5 μ [3, 4]. This broad spectrum of lysosomes was fractionated by Straus [3], in his classic study, into three subclasses by successive differential pelleting of kidney cortical homogenates. This work and subsequent studies on the distribution of acid hydrolases in the kidney subcellular fractions [3, 5–11] have also demonstrated considerable heterogeneity of the lysosomes with respect to enzyme content. This heterogeneity of lysosomal enzymes has also been observed by histochemical studies [12, 13] and has been attributed to different physiological functions of the various cell types of the nephron [14].

Cells of the proximal tubule contain uniquely large (2 to 5 μ) lysosomes [3, 6, 15] in agreement with the large lysosomal volume in the cells of the proximal tubule segments [16] being very similar to the distribution of acid hydrolases in this region of the nephron [2]. The involvement of these large lysosomes in the catabolism of proteins taken up from the glomerular filtrate has been demonstrated by histochemical staining [17] showing that the uptake and breakdown of horseradish peroxidase was maximal in the lysosomes of the proximal convoluted tubule. The complement of lysosomal enzymes [18, 19] in these lysosomes most probably reflect their specialized function. Although it is evident that cortical homogenates contain other lysosomes, it is the protein droplets which have been most extensively characterized [12, 18] and commonly isolated [3, 5–7, 11].

The presence of acid hydrolases in both the cortical mitochondrial/lysosomal and the microsomal fraction [10, 20] illustrates the very broad population of small lysosomes (0.1 to 1.5 μ) which appear to contain primary lysosomes, endocytic vesicles, precursor vesicles to the protein droplets [6, 11], renin granules [21, 22] and other acid hydrolase-containing organelles. This heterogeneous mixture of subcellular particles has not been studied in any great detail, possibly since it is so difficult to subfractionate [23]. Some of the populations of smaller lysosomes may originate from the distal tubule, since the high acid hydrolase activity in this region of the nephron [2] in relation to the low lysosomal volume [16] suggests that lysosomes of the distal tubule may be extremely rich in lysosomal enzymes. Much of our present knowledge of the properties and enzyme composition of small lysosomes stems indirectly from the attempts to purify renin containing granules [24–27] and from subcellular fractionation of cortical homogenates by differential pelleting.

We report here a systematic study providing reliable distribution profiles of cortical lysosomal populations, which have been further fractionated by rate and isopycnic sedimentation and characterized by employing a comprehensive range of marker enzymes, use of labelled proteins as probes for various lysosomal populations, and by morphological examination.

Received for publication February 15, 1985
and in revised form February 19 and October 1, 1986

Methods

Tissue homogenization and fractionation

Male Wistar rats (150 to 180 g), starved overnight, were anesthetized with ether and their kidneys were removed. Entire cortices (including deep cortex) from eight to 12 rats were carefully dissected free, and homogenized in 0.25 M sucrose/5 mM Tris-HCl, pH 7.4, using a Potter Elvehjem, type C homogenizer (A. H. Thomas, Philadelphia, Pennsylvania, USA) power driven at 1000 rev/min. The 10% (wt/vol) homogenate was filtered through a coarse sieve (tea strainer). Examination by phase microscopy of the initial homogenate and the debris collected on the filter indicates that most of the glomeruli remain intact and are removed by the filter. The cortical homogenate thus appears to be relatively enriched in tubular material.

The homogenate was centrifuged at 1,115 g · min (2,000 rev/min for 3 min) to sediment the nuclear fraction (N). This was resuspended, centrifuged again, and the combined supernatants were spun at 27,530 g · min (10,000 rev/min for 3 min) to pellet the mitochondrial/lysosomal (ML) fraction. This was found to improve the recovery of lysosomes in the ML fraction. After centrifuging the post ML supernatant at 7,950,000 g · min (43,000 rev/min for 60 min) the microsomal pellet (MIC) and the supernatant (SUP) were obtained.

Density gradient centrifugation

The ML fraction was resuspended in the isotonic buffered sucrose and further subfractionated by rate sedimentation in an HS zonal rotor (MSE Scientific Instruments, Crawley, Sussex, UK) containing 550 ml of exponential sucrose (with 5 mM Tris HCl, pH 7.4) gradient ranging from 0.5 M to 1.7 M and 150 ml 2 M sucrose as the cushion. The exact profile of the gradient is presented in Figure 1. After loading the ML fraction, the zonal rotor was spun at 8,000 rev/min for one hour. Finally, 20 ml fractions were collected for the various assays.

Two different regions of the rate zonal spin (Fig. 1), the slow sedimenting, small lysosomes and rapidly sedimenting, large lysosomes were further fractionated by isopycnic banding. The chosen fractions were pooled and the sucrose concentration adjusted to the lowest gradient concentration. This sample was loaded into a B 14 zonal rotor (MSE Scientific Instruments) containing a linear sucrose gradient whose exact profiles and concentrations are indicated in Figures 3 and 4. The rotor was then spun at 45,000 rev/min for approximately 16 hours.

The microsomal fraction was subfractionated by isopycnic flotation using the method of Norris et al [28] which was slightly modified with the linear sucrose gradient being extended to cover the range 0.7 to 2.3 M sucrose (Fig. 5). The microsomes were resuspended in 2 M sucrose and laoded into the rotor at precisely the 2 M point of the gradient.

Chemical and enzyme assays

Protein was measured either by the protein-dye binding method described by Bradford [29] or by an automated adaptation [30] of the Lowry et al method [31] modified to include a sodium hydroxide digestion step [32]. Bovine serum albumin was used as standard in both methods. Total RNA was determined as described by Fleck and Begg [33] while hydroxyproline was estimated as described by Nobbs, Walker and Davies [34]. Various marker enzymes [35] were assayed as prescribed in the following references. Succinic dehydrogenase (SDH) was determined by the method of Pennington [36] while monoamine oxidase (MAO) was assayed using a fluorimetric adaptation [37] of the method described by Weissbach et al [38]. Catalase was determined as described by Leighton et al [39]. Alkaline phosphatase was assayed in the presence of divalent metal ions [40] and potassium fluoride was added to inhibit acid phosphatase activity [41]. Na-K-stimulated Mg-dependent ATPase was assayed using assay procedure A described by Schwartz et al [42]. Na-K-ATPase activity was calculated as the difference of total ATPase minus basal Mg-ATPase. Cathepsin D was assayed as described by Barrett [43] while N-acetyl-β-D-glucosaminidase (NAG) was assayed as described by Hultberg and Öckerman [44]. Alkaline phosphodiesterase, acid phosphodiesterase, acid β-galactosidase and acid β-glucuronidase were all assayed by a manual adaptation of the automated assay described by Hinton and Norris [32]. p-Nitrophenyl-β-glucuronide was used as substrate in the acid β-glucuronidase assay. Acid ribonuclease (acid RNase) was assayed as described by Dobrota, Burge and Hinton [45]. Acid phosphatase was measured with β-glycerophosphate as the substrate [45]. This enzyme was also used as an index of lysosomal latency (a ratio of bound/total acid hydrolase activity) by measuring total activity (in the presence of 0.0016% (wt/vol) digitonin) and expressing latency as: Total-Free/Total × 100. 5'-Nucleotidase was assayed in the presence of 0.01 M tartrate to suppress acid phosphatase activity. The assay was performed essentially as described by El-Aaser and Reid [46] using a 0.1 M Tris-HCl pH 7.8 buffer containing 5 mM magnesium chloride. Glucose-6-phosphatase was also assayed in the presence of tartrate as described by Dobrota and Hinton [47]. Renin activity was determined by the radioimmunological microassay based on angiotensin I trapping by antibody [48].

Lowry protein values and enzyme activities were corrected for sucrose inhibition as previously reported [49].

Labelling of lysozyme with ^{125}I

Lysozyme (Boehringer, Mannheim, West Germany) was labelled with NaI^{125} (Amersham International Ltd, Amersham, Bucks, UK) by the Iodogen procedure [50]. Twenty μg of lysozyme (0.5 mg/ml) was labelled with 800 μCi NaI^{125} and the labelled protein was purified by passage through Sephadex G-25. After addition of unlabelled lysozyme each rat was injected intravenously via the jugular vein 30 minutes before sacrifice, with 0.2 ml of lysozyme containing approximately 1 mg and 20 μCi ^{125}I. A total of four rats were used per experiment.

Labelling of Cd-thionein with ^{109}Cd

Hepatic Cd-Zn-thionein isolated from male Wistar rats [51] was labelled with $^{109}CdCl_2$ (Amersham International Ltd.). The thionein was incubated from 16 hours at 4°C with carrier-free $^{109}CdCl_2$ (1.5 mg thionein, 50 μCi ^{109}Cd) and purified by chromatography on Sephadex G-25 [52]. The labelled protein was injected intravenously via the jugular vein (5 μCi, 0.2 mg thionein per animal), 30 minutes before sacrifice [52, 53]. A total of four rats were used per experiment.

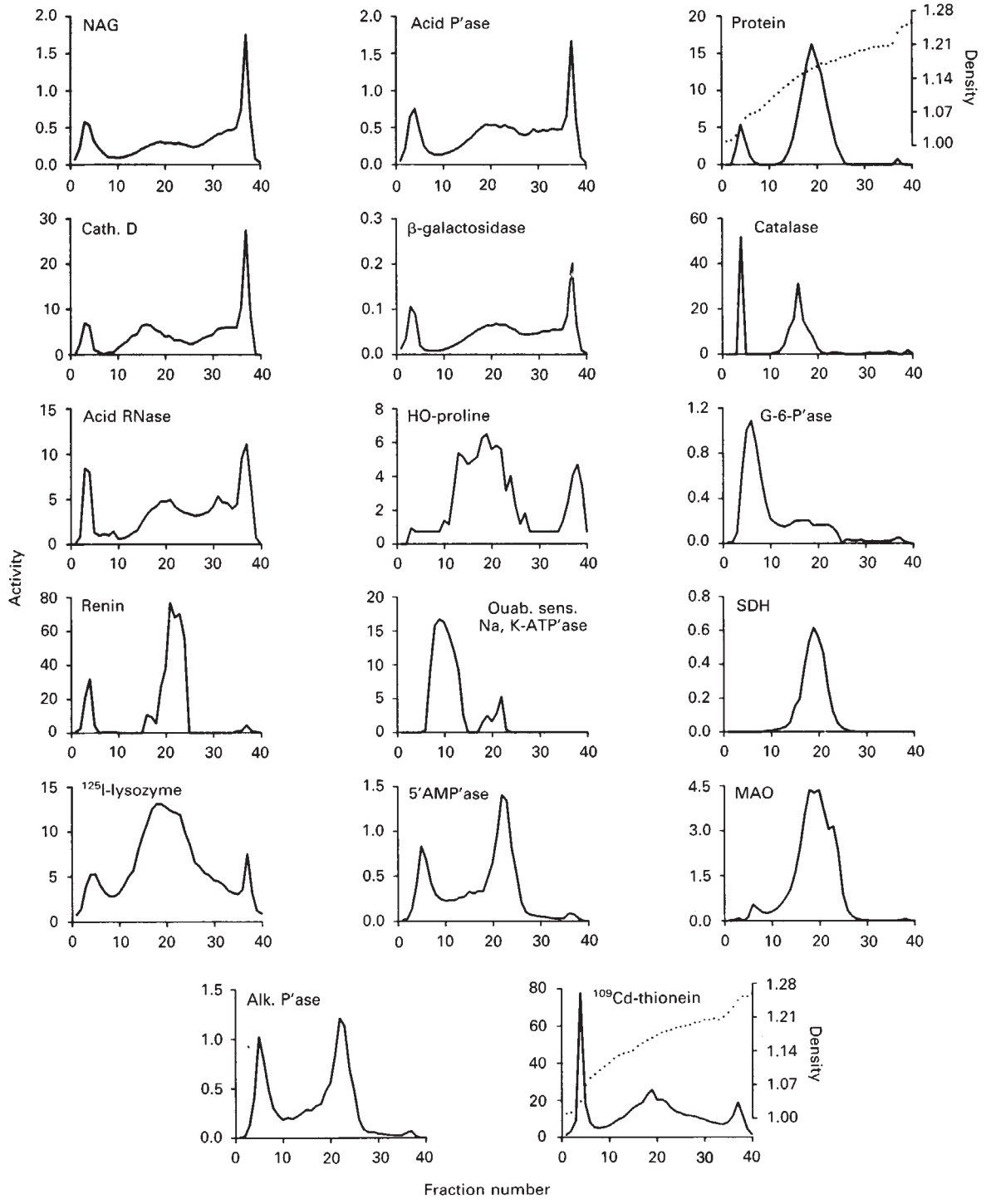

Fig. 1. *Distribution of marker enzymes, protein, ^{125}I label and ^{109}Cd label after rate sedimentation of a rat kidney cortex ML fraction in a zonal HS rotor.* The rotor contained a 550 ml exponential sucrose gradient ranging 0.5 M to 1.7 M and 150 ml of 2 M sucrose as the cushion. After loading the resuspended ML fraction into the rotor it was spun at 8000 rev/min for 1 hour. Protein is presented as mg/fraction, succinic dehydrogenase (SDH), monoamine oxidase (MAO), glucose-6-phosphatase (G-6-Pase), 5'-nucleotidase (5' AMP'ase), acid β-glycerophosphatase (acid Pase), acid β-galactosidase, N-acetyl-β-D-glucosaminidase (NAG), Na-K-ATPase and alkaline phosphatase (alk. P'ase) are given as μmol/min (IU) per fraction; renin as 10^{-5} μmol, angiotensin I formed/60 min/fraction; catalase values are units/min/fraction [39]. Acid ribonuclease (acid RNase) units are ΔA_{260}/min/fraction and cathepsin D ΔA_{280}/min/fraction. ^{125}I-lysozyme is given as cpm/0.001 ml and L-hydroxyproline as μmol/fraction. The distribution of ^{109}Cd-thionein, which is from a different experiment, is given as cpm/0.005 ml.

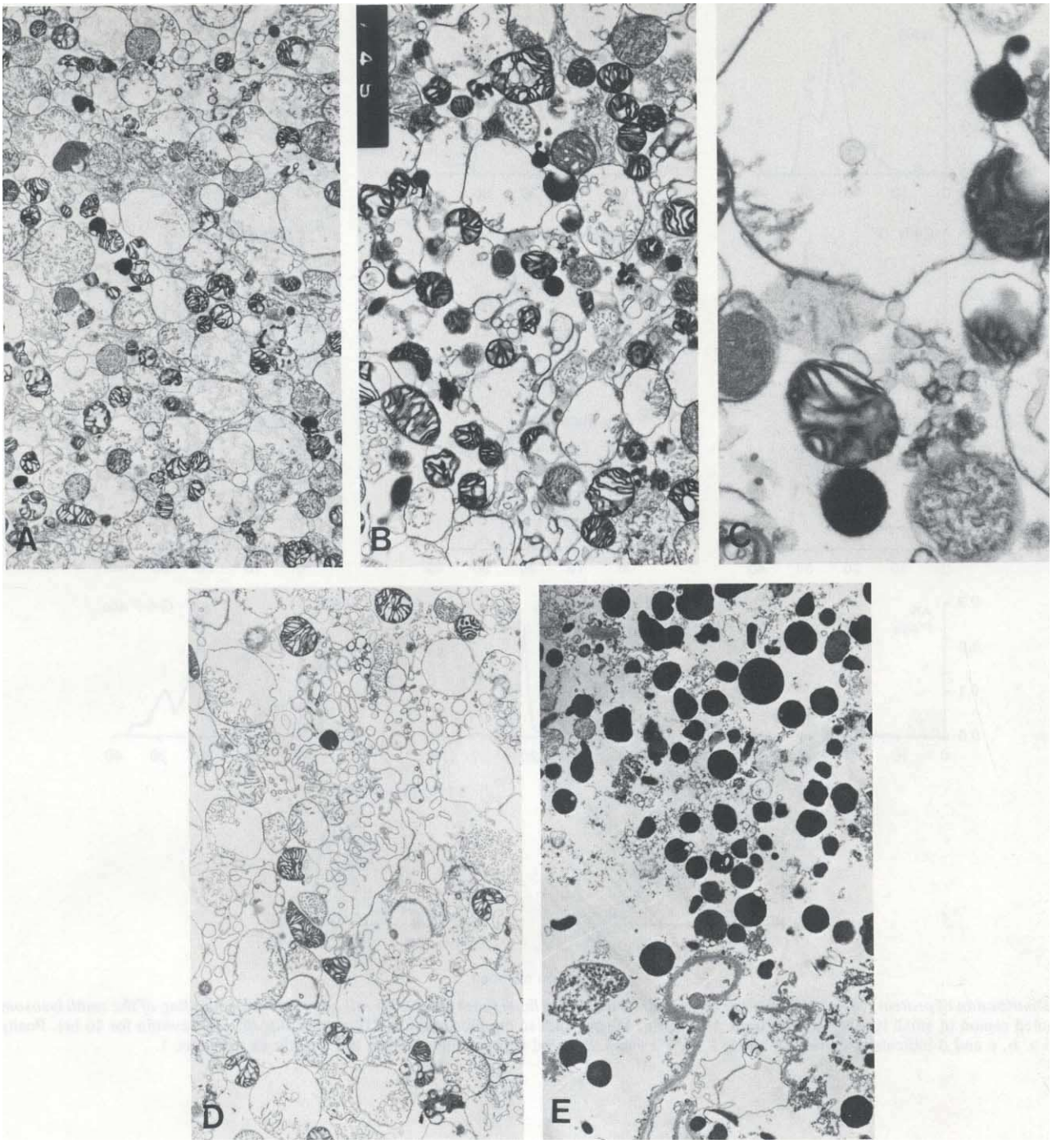

Fig. 2. *Morphology of lysosomes and other structures present in three regions of the cortical ML fractionated by rate sedimentation.* **A.** fractions 11-20 × 4,700; **B.** fractions 11-20 × 10,080; **C.** fractions 11-20 × 30,880; **D.** fractions 21-25 × 3,270; **E.** fractions 36-39 × 2,030. Fraction numbers refer to those illustrated in Figure 1.

Labelling of Cd-thionein with ^3H

Liver Cd-Zn-thionein was labelled with N-Succinimidyl-[2,3-^{3}H]propionate (Amersham International Ltd, Amersham, Bucks, UK), according to the method described by Tang, Davis and Kitcher [54] and purified by chromatography on Sephadex G-25. Each animal (5 per experiment) was injected with 2 μCi, 0.2 mg thionein. This Cd-thionein, which is labelled with ^{3}H on the protein molecule (as ^{3}H propionate on lysine residues) was used for time course uptake and catabolism studies in preference to the ^{109}Cd-thionein because the Cd is susceptible to removal by acidification.

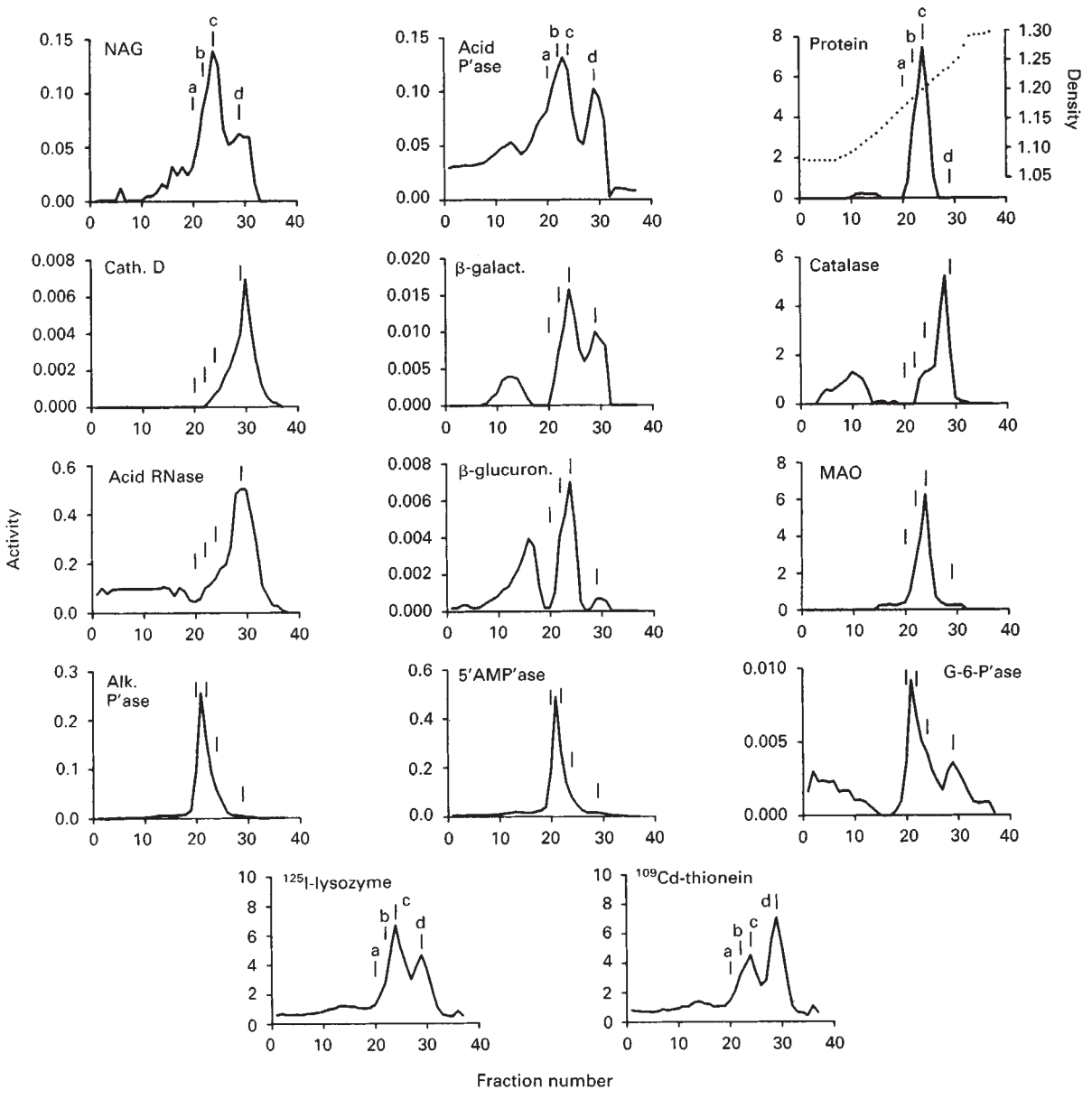

Fig. 3. *Distribution of protein, various marker enzymes and ^{125}I and ^{109}Cd label (both cpm/0.005 ml) after isopycnic banding of the small lysosomes.* The pooled region of small lysosomes (fractions 11-24, Fig. 1) was spun to equilibrium in a B14 zonal rotor (45,000 rev/min for 16 hr). Positions marked a, b, c and d indicate densities of 1.165, 1.18, 1.2 and 1.235 g/ml respectively. Details are exactly as in Figure 1.

Electron microscopy

Gradient fractions were fixed by firstly diluting out the sucrose to approximately isotonic followed by, adding in the cold with continuous stirring, sufficient concentrated glutaraldehyde to give a final concentration of 2%. After fixing for one to two hours, the particulate material was spun down (approx. 100,000 × g for 40 min). The pellets were washed several times with 0.1 M Na-cacodylate, pH 7.4, and postfixed for one hour in 2% OsO_4. The samples were then dehydrated in the alcohol series, transferred into propylene oxide, then propylene oxide/EPON mixture (1:1) and finally embedded in EPON 812 (Taab Laboratories, Reading, Berks., UK). Silver grey sections (60 nm thick) were cut, counterstained and examined with a JEOL 100B microscope (JEOL, Tokyo, Japan).

Results

After a careful survey of the known characteristics of kidney lysosomes [3, 5, 8, 12, 20], it was decided to retain the g · min value of 27,530 (10,000 rev/min for 3 min), as recommended by Maunsbach [12, 15] for pelleting the kidney ML fraction because this gave a good compromise between lysosomal recovery (an average of 41% of the total homogenate acid hydrolase content) [12, 55] and contamination by other organelles, and

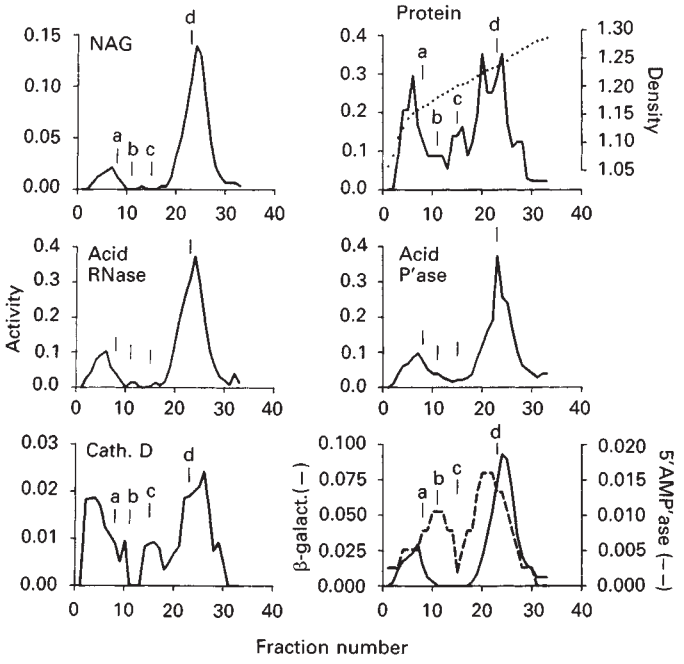

Fig. 4. *Distribution patterns of protein and marker enzymes after isopycnic banding of the large lysosomes (protein droplets) recovered from the rate zonal spin.* Fractions 36-39 (Fig. 1) were pooled and spun to equilibrium under exactly the same conditions as in Figure 3. Protein and enzyme activities are as described in Figure 1 and the density reference points as in Figure 3.

also permitted the direct comparison with published data on the ML fraction.

Very gentle homogenization has been recommended for renal tissue [9] as there have been conflicting reports on both fragility and latency of kidney lysosomes [8, 9, 56, 57]. While examining the optimal homogenization conditions we found that there was no increase in free activity (as a percentage of total activity) of β-glycerophosphatase, NAG or β-galactosidase in the homogenate from two to five strokes, indicating that cortical lysosomes are rather resistant to mechanical disruption.

The proportions of acid hydrolases, other marker enzymes and protein are presented in Table 1 as percent distribution (yield) in the kidney cortical fractions. Specific activities (SA) of the enzymes measured, Table 2, are consistent with previously quoted values.

Rate sedimentation of cortical lysosomes

The approach adopted to examine the cortical lysosome populations was to subfractionate the whole ML fraction by rate zonal sedimentation. The distribution of lysosomal and other marker enzymes, in a typical rate sedimentation, are shown in Figure 1. When expressed as a percentage of the ML fraction (or sample loaded onto the zonal rotor), the percentage of protein recovered in all the fractions of the rate zonal spin was 96.9%. The lowest value for the marker enzymes was 76.6%, obtained for glucose-6-phosphatase, while the highest was 102.0%, obtained for acid β-galactosidase.

From the protein pattern it is evident that three distinct regions can be identified: a) The peak at fraction 3-4 (6.8% of protein recovered) represents the initial sample position and hence contains the soluble protein and very small particles which do not sediment under these conditions; b) this is the major protein peak (fractions 13-25; 88.2% of the protein) which is composed of mitochondria, brush border membranes, small lysosomes, etc; c) the most rapidly sedimenting material (0.7% of the protein), found in fractions 36-37, contains large lysosomes.

The distribution of lysosomal enzymes show three essential features. First, each of the acid hydrolases assayed are present in the peak of large lysosomes (fractions 36-37). The much broader band of smaller lysosomes (fractions 13-25), which contain acid phosphatase, β-galactosidase and NAG, appear to sediment faster than the small lysosomes which contain acid RNase and cathepsin D. The cathepsin D peak, at fraction 14-18, indeed seems to be associated with the smallest (slowest sedimenting) lysosomes. Renin shows a distribution different from the main peak (fractions 20-24) sedimenting with the fastest sedimenting band of small lysosomes.

In the experiment illustrated in Figure 1, the animals had received an intravenous injection of ^{125}I labelled lysozyme. The distribution of ^{109}Cd-thionein from a different experiment is also illustrated in Figure 1 to compare the distributions of the two different proteins. Both the ^{125}I and the ^{109}Cd patterns indicate that the label is present in the large lysosomes region (fractions 36-37) and that a large proportion (52.2% of ^{125}I and 43.3% of ^{109}Cd) is also associated with smaller lysosomes (fraction 13-25). The difference between the ^{125}I and ^{109}Cd patterns in the first peak (fractions 2-6) is due to the larger proportion of ^{109}Cd-thionein found in the cytosol. The high peak of ^{109}Cd thus represents soluble material which does not sediment away from the original sample position. The ^{125}I in all the fractions was acid (TCA) precipitable and thus was not due to free or released ^{125}I.

The time course of uptake and processing of ^{3}H labelled Cd-thionein is summarized in Table 3. The maximal amount of label in the cortex, expressed as a percent of injected dose, at 10 minutes after injection indicates very rapid uptake and binding followed by a decrease, which suggests rapid catabolism. The ^{3}H label in the ML fraction, as a percent of total in the cortex, increases steadily to 80% at 1.5 hours. The maximal proportion of microsomal label at 10 minutes followed by a decrease suggest a transfer from small vesicles to larger particulates. This is also consistent with the relative decrease in the percent of small lysosomes (as percent of ML) with time and an increase in the percent of ^{3}H in the large lysosomes.

The main mitochondrial peak, shown by the pattern of SDH and MAO, is in tubes 16-22 while the peroxisomes represented by the broad peak of catalase, between fractions 12 and 19, sediment somewhat slower. The large peak of catalase in the soluble regions indicates either the presence of cytosol-located enzyme [58] or solubilized enzyme released from disrupted peroxisomes. The glucose-6-phosphatase region (tube 20) coinciding with the SDH peak suggests association of the ER with mitochondria, as previously reported [59]. Alkaline phosphatase and 5′-nucleotidase show similar distributions and are recovered as a well defined band around fraction 22, while the major part of Na-K-stimulated ATPase (basolateral membrane) is recovered around fractions 10-11. The other peak of alkaline phosphatase and 5′-nucleotidase at fractions 4-6 is most prob-

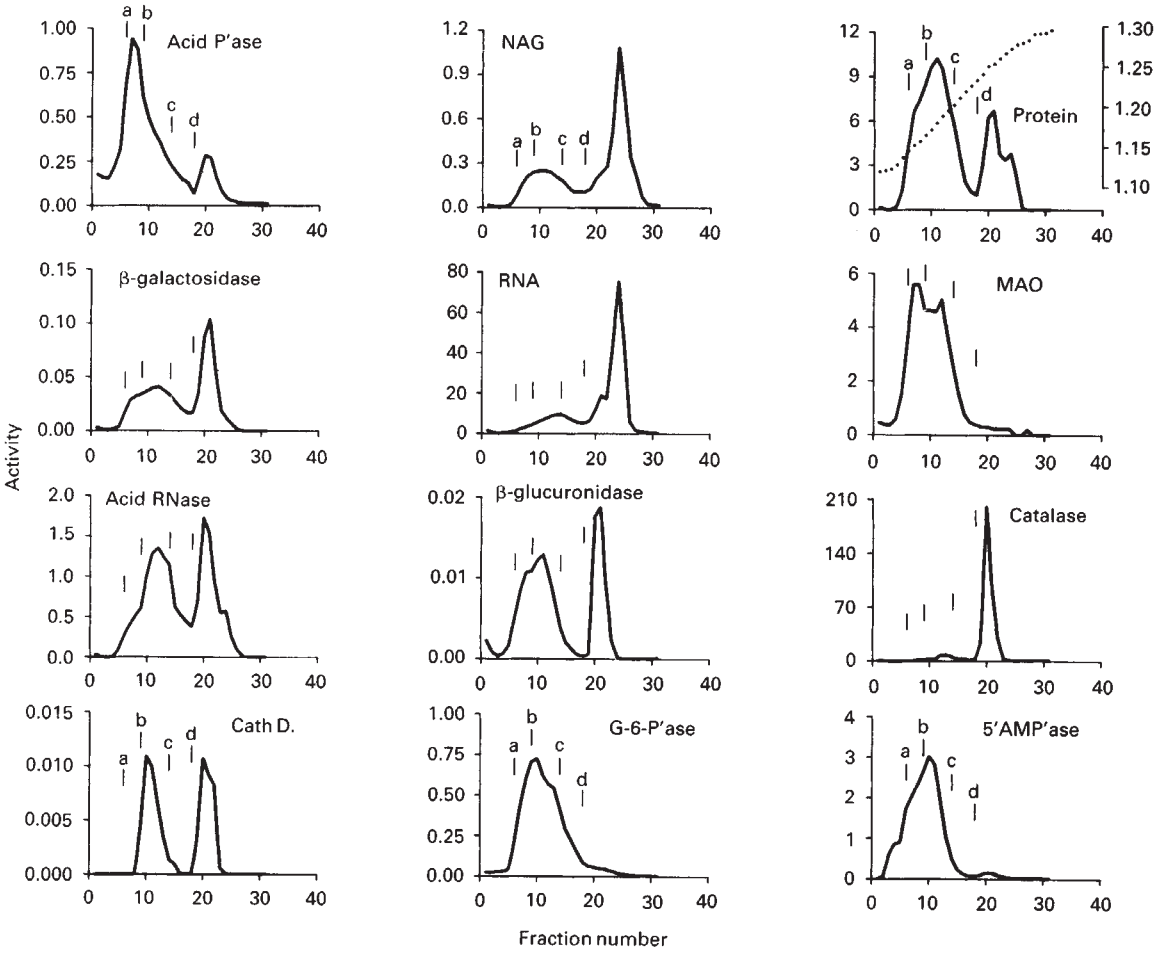

Fig. 5. *Distribution of protein, RNA and marker enzymes after isopycnic flotation of cortical 'microsomes' which were resuspended in 2 M sucrose and loaded under a linear sucrose gradient (0.7–2.3 M) in a B14 zonal rotor.* A cushion of 2.3 M was used to push the sample band away from the edge wall of the rotor. The rotor was spun at 45,000 rev/min for 16 hr. Protein and enzyme activities are as described in Figure 1; RNA is μg/fraction and positions a, b, c and d are densities of 1.145, 1.165, 1.202 and 1.234 g/ml, respectively.

ably due to small brush border vesicles which do not sediment under the conditions employed.

The morphology of the subcellular structures, recovered from three important regions of the rate sedimentation spin of the ML fraction is presented in Figure 2. The structure of material found in the slow sedimenting region (fractions 13-18) of the broad band of small lysosomes shows among numerous mitochondria and other membranous elements small but significant numbers of electron dense small granules. A fairly–low power view shows at least 10 structures which are electron dense and most probably represent lysosomes (Fig. 2A). At a slightly higher magnification (Fig. 2B) a few small lysosomes can be identified by the homogeneous electron dense appearance. Figure 2c illustrates a region of two lysosomes in plate b which has been further magnified to show the electron dense structures so characteristic of lysosomes. Rather shrunk damaged mitochondria are seen in plates A, B, C and D (Fig. 2). The second region (Fig. 2D), enriched in 5-nucleotidase and alkaline phosphatase and representative of brush border membrane (fractions 19-23, Fig. 1) contains numerous large, smooth membrane fragments. The possibility that some of these may be swollen mitochondria was investigated by assaying the distribution of MAO-located mainly on the inner face of the mitochondria outer membrane [60]. The pattern of MAO shown in Figure 1 corresponds reasonably well with that of the normal mitochondrial marker enzyme SDH. The large membranous sacks seen in plate D (Fig. 2) therefore most likely represent sheets of brush border encircling vesiculated microvilli.

The morphology of the rapidly sedimenting band of large lysosomes (fractions 35-37) is shown in Figure 2E. The predominant structures are the large lysosomes, which are very similar to those found in the proximal tubule cells [9, 15]. From the absence of any marker enzymes, other than the acid hydrolases, from this band it appears that only lysosomes are present. However, morphology shows small amounts of two distinct contaminants: sheets of smooth membranes and fragments of tubular basement membrane (BM). The large membrane sheets appear to be firmly attached to the basement membrane and thus probably represent fragments of the basolateral plasma membrane, although the specific marker enzyme, Na-K-ATPase [61] was not detected (Fig. 1). The presence of basement membrane material in the large lysosome region was checked by assaying one convenient chemical marker, L-hydroxyproline [62]. The high proportion (6.8%) of L-

Table 1. Percent distribution yield in the classical subfractions of the rat kidney cortex

	N	ML	MIC	SUP	Total
Protein	16.9 ± 2.8 (19)	26.7 ± 3.7 (18)	19.3 ± 2.9 (18)	37.0 ± 2.2 (16)	94.1 ± 6.4 (16)
Succinic dehydrogenase	11.2 ± 2.4 (15)	65.1 ± 6.8 (18)	14.9 ± 3.1 (17)	5.8 ± 2.0 (17)	98.9 ± 10.1 (16)
Monoamine oxidase	21.0 (1)	42.5 (1)	13.4 (1)	1.5 (1)	78.4 (19)
Catalase	8.0 ± 4.5 (8)	16.1 ± 6.8 (8)	6.4 ± 1.7 (8)	76.9 ± 5.2 (10)	97.4 ± 14.0 (8)
Glucose-6-phosphatase	19.2 ± 4.1 (18)	19.9 ± 4.2 (23)	52.2 ± 4.4 (17)	7.1 ± 2.2 (18)	95.8 ± 5.4 (22)
5′-nucleotidase	16.0 ± 2.9 (19)	22.5 ± 5.1 (15)	44.1 ± 7.4 (14)	12.3 ± 2.7 (20)	94.2 ± 6.4 (18)
Alkaline phosphatase	17.4 ± 3.7 (8)	26.5 ± 7.9 (6)	35.0 ± 13.5 (7)	14.6 ± 5.4 (7)	92.0 ± 8.7 (8)
Alkaline phosphodiesterase	16.4 ± 1.7 (6)	21.6 ± 4.8 (6)	42.0 ± 4.0 (6)	18.0 ± 3.7 (6)	98.3 ± 14.1 (6)
Acid phosphatase	12.0 ± 2.7 (20)	33.8 ± 6.1 (20)	19.5 ± 3.5 (19)	33.2 ± 7.1 (18)	96.5 ± 7.3 (20)
β-galactosidase	15.0 ± 2.1 (20)	40.3 ± 4.3 (20)	11.8 ± 2.0 (18)	32.3 ± 3.2 (21)	95.2 ± 5.5 (19)
NAG	21.7 ± 2.5 (18)	44.0 ± 4.3 (18)	24.2 ± 2.5 (18)	8.5 ± 3.2 (15)	93.9 ± 8.6 (18)
Cathepsin D	18.4 ± 3.2 (10)	44.7 ± 3.5 (10)	23.2 ± 4.8 (10)	17.0 ± 4.4 (7)	91.0 ± 5.5 (7)
Acid RNase	19.2 ± 3.1 (9)	43.6 ± 5.8 (10)	9.8 ± 1.6 (9)	23.3 ± 5.2 (9)	92.8 ± 11.5 (9)
Acid phosphodiesterase	16.9 ± 3.4 (11)	22.1 ± 4.1 (11)	35.6 ± 5.7 (13)	33.1 ± 1.3 (6)	90.2 ± 11.2 (10)
Renin	20.2 ± 5.8 (9)	41.3 ± 6.3 (9)	3.0 ± 0.4 (9)	32.9 ± 4.8 (7)	99.6 ± 12.0 (8)
Lysozyme (^{125}I)	15.0 (1)	34.4 (1)	29.5 (1)	4.7 (1)	84.0 (1)
^{109}Cd-thionein	16.4 (1)	21.5 (1)	13.1 (1)	37.6 (1)	98.4 (1)
Hydroxyproline	87.2 (1)	10.5 (1)	0.9 (1)	1.1 (1)	99.7 (1)

Yield is presented as the mean percentage of the total homogenate value ± SD, with the number of observations in parenthesis. Abbreviations are: the fractions N (Nuclear), ML (Mitochondria/lysosomes), MIC (Microsomes) and SUP (Cytosol or supernatant).

Table 2. Specific activities of marker enzymes in the homogenate (H) and classical fractions of rat kidney cortex

	H	N	ML	MIC	SUP
Succinic dehydrogenase	0.0149 ± 0.0032 (16)	0.0115 ± 0.0044 (15)	0.043 ± 0.017 (16)	0.0124 ± 0.0043 (13)	0.0018 ± 0.0005 (14)
Monoamine oxidase	0.216 (1)	0.356 (1)	0.393 (1)	0.223 (1)	0.009 (1)
Catalase	1.15 ± 0.32 (11)	0.66 ± 0.25 (8)	0.77 ± 0.22 (10)	0.49 ± 0.13 (8)	2.8 ± 1.0 (11)
Glucose-6-phosphatase	0.088 ± 0.016 (23)	0.119 ± 0.034 (21)	0.074 ± 0.018 (21)	0.272 ± 0.053 (19)	0.022 ± 0.005 (14)
5′-nucleotidase	0.119 ± 0.021 (23)	0.125 ± 0.029 (21)	0.086 ± 0.016 (21)	0.289 ± 0.09 (17)	0.0385 ± 0.009 (22)
Alkaline phosphatase	0.143 ± 0.045 (11)	0.113 ± 0.05 (8)	0.125 ± 0.44 (11)	0.394 ± 0.16 (9)	0.043 ± 0.016 (10)
Acid phosphatase	0.079 ± 0.015 (16)	0.073 ± 0.024 (15)	0.122 ± 0.033 (19)	0.086 ± 0.023 (16)	0.071 ± 0.019 (19)
β-galactosidase	0.0116 ± 0.001 (19)	0.0111 ± 0.003 (19)	0.0208 ± 0.0068 (16)	0.0074 ± 0.0017 (16)	0.0106 ± 0.004 (16)
NAG	0.084 ± 0.013 (18)	0.118 ± 0.027 (16)	0.152 ± 0.029 (17)	0.131 ± 0.029 (17)	0.021 ± 0.01 (16)
Cathepsin D	0.0057 ± 0.002 (10)	0.007 ± 0.0018 (7)	0.0105 ± 0.0025 (10)	0.0077 ± 0.003 (10)	0.0049 ± 0.003 (8)
Acid RNase	0.135 ± 0.035 (9)	0.142 ± 0.032 (9)	0.196 ± 0.066 (10)	0.064 ± 0.029 (10)	0.098 ± 0.025 (9)
Acid phosphodiesterase	0.0047 ± 0.0015 (13)	0.0053 ± 0.0022 (11)	0.0049 ± 0.0016 (11)	0.0103 ± 0.004 (10)	0.0035 ± 0.0014 (11)
Renin	0.0068 ± 0.0034 (10)	0.0118 ± 0.0054 (9)	0.011 ± 0.0028 (8)	0.00105 ± 0.0005 (10)	0.0065 ± 0.003 (9)

Specific activities are as the mean ± SD with the number of observations in parenthesis. Specific activities are: acid RNase is ΔA_{260}/min/mg protein; for cathepsin D ΔA_{280}/min/mg protein; catalase units/min/mg protein; all other enzymes are μmol/min/mg protein.

Table 3. Time course of uptake and processing of ^{3}H labelled co-thionein

	% of injected		% of homogenate				% of ML	
	plasma	cortex	N	ML	MIC	SUP	Small lysosomes	Large lysosomes
10 min	11.6	25.0	11.4	25.7	26.4	22.7	53.8	3.5
30 min	5.6	14.8	16.8	31.1	16.0	47.4	43.3	9.1
1.5 hr	1.73	2.7	18.7	79.8	0	0	40.9	26.4
24 hr	0.43	0	0	0	0	0	0	0

The plasma percent value is calculated on the basis of 8 ml total plasma volume per rat. The percentage of ^{3}H in small and large lysosomes represent total summed activities present in the peaks of these two regions following rate sedimentation of the cortical ML fraction. These peaks were carefully chosen after checking the distributions of the acid hydrolase peaks and correspond to fractions 12-26 (small lysosomes) and 35-39 (large lysosomes) illustrated in Figure 1.

hydroxyproline in relation to the low protein content (0.7%) of the protein droplet band suggests that this region contains a significant proportion of BM material in the ML fraction.

Although slightly contaminated by BM and smooth membranes, this region of protein droplets (fractions 35-37 in Fig. 1) contains highly purified lysosomes, as judged by the RSA of 41 ± 17 obtained as an average of 5 of the acid hydrolases measured in 16 experiments. The highest purifications which have been achieved previously for these lysosomes are between 10- and 20-fold [3, 8, 10, 12].

Equilibrium banding of the two major lysosomal populations

Small lysosomes. The small lysosome region (fractions 13-25) of the rate sedimentation spin (Fig. 1) was pooled and spun to

equilibrium in sucrose gradients. This was done first to identify the banding densities of the small lysosomes and second to demonstrate the separate identity of the lysosomes from the other organelles, which are the major components of this region. The enzyme profiles of the equilibrium spin are shown in Figure 3 with the most important bands (1.165, 1.18, 1.20 and 1.235 g/ml) indicated.

Mitochondrial marker enzymes SDH (not illustrated) and MAO were found at a rather high density of 1.20, suggesting some mitochondrial damage caused by hydrostatic pressure and hypertonicity of the gradient. The 5′-nucleotidase and alkaline phosphatase indicating the position of the plasma membrane (brush border) are located at a density of 1.175, which is rather high for smooth membranes (normally 1.165 g/ml). The glucose-6-phosphatase pattern marks the endoplasmic reticulum (ER), which shows the classic bimodal distribution [63] of smooth ER (density 1.16 to 1.18 g/ml) and rough ER (1.19 to 1.24 g/ml). The distinct peak of catalase at density 1.23 is consistent with the established equilibrium banding of peroxisomes [39].

The patterns of acid hydrolases are somewhat variable, banding at three densities which probably represent lysosomal fragments (1.11 to 1.15 g/ml), light lysosomes (1.20 g/ml) and dense lysosomes (1.235 g/ml). The acid hydrolase activity at the low density is essentially non-latent and this suggests that this band contains lysosomal membranes or possibly smooth membrane vesicles which have picked up released lysosomal enzymes. While the peak at density 1.20 g/ml is consistent with the density of "normal" lysosomes of the liver and other tissues [64], the dense peak at 1.235 g/ml is the same as reported for the kidney droplets [6, 15] and is confirmed in the equilibrium spin of the large lysosomes (Fig. 4). Both these peaks show latency, demonstrating the presence of intact lysosomes. The presence of acid RNase and cathepsin D mainly in the region banding at density 1.235, confirms the heterogeneous distribution of acid hydrolases among the various lysosomal populations fractioned by rate sedimentation (Fig. 1).

The distributions of ^{125}I-lysozyme and ^{109}Cd-thionein indicate that these two proteins are present in normal (light) and dense lysosomes and not in smooth membrane fragments (density 1.16 to 1.18 g/ml) when injected 30 minutes prior to sacrifice.

Large lysosomes—protein droplets. The large, rapidly sedimenting lysosomes obtained after the rate zonal spin of the ML fraction were pooled and subjected to equilibrium banding under exactly the same conditions as employed for the small lysosomes. The distribution of protein, density and marker enzymes are illustrated in Figure 4. The acid hydrolases, acid phosphatase, β-galactosidase, NAG, RNase and cathepsin D all show almost identical patterns with one single peak at density 1.235 g/ml, which is consistent with previously reported data on the kidney's large, dense lysosomes [6, 15]. No ^{125}I lysozyme or ^{109}Cd-thionein profiles are shown because neither of the labelled proteins were used in the experiment illustrated in Figure 4. However, in experiments when these labelled proteins were administered, the label was present as a single peak at density 1.235 g/ml. All the acid hydrolases show released activity in the sample region (fractions 1-8, Fig. 4), which suggests some fragility of the kidney droplets.

Small lysosomes and lysosomal fragments recovered in the microsomal fraction

The high recovery of lysosomal acid hydrolases in the microsomal fraction (10 to 36%; Table 2) is not surprising since the ML fraction is pelleted at a rather low g · min value as recommended by Maunsbach [12] for the preparation of large kidney granules. As also noted by other workers [8–10], the microsomal fraction clearly contains a significantly high proportion of small lysosomes.

The distribution of various marker enzymes after equilibrium flotation of the microsomal fraction are shown in Figure 5. Acid phosphatase shows some activity in the sample region (density 1.25 g/ml) and appears as a large peak of non-latent activity at density 1.15 with a shoulder of latent activity in the density range 1.19 to 1.20. The patterns of β-galactosidase, β-glucuronidase, acid RNase, cathepsin D and NAG demonstrate significant activity in the sample region and a broad band of activity in the density range 1.15 to 1.20 with subtle, but distinct variations in the distribution of all the hydrolases. Latency is observed in the density range 1.19 to 1.20 demonstrating the presence of intact lysosomes (microsomal, small light), while acid hydrolase activity (free or non-latent) at lower densities represents fragments of lysosomal membranes, and released acid-hydrolase activity associated with smooth membrane vesicles. The activity recovered in the sample region is most probably due to released activity and not dense lysosomes because there are no signs of any of the enzymes banding at densities 1.23 to 1.24, indicating the absence of intact dense lysosomes from the microsomal fraction.

The sharp, large peak on NAG at density 1.27 g/ml is not due to released enzyme in the sample region, but is caused by its association with free ribosomes as previously noted [9] and confirmed here by the virtually identical distribution of RNA.

The rather broad band of glucose-6-phosphatase marks the position of smooth ER (1.14 to 1.17 g/ml) with a shoulder of the enzyme at higher densities (1.20 g/ml) attributable to rough ER vesicles as confirmed by the pattern of RNA. The plasma membrane marker enzyme, 5′-nucleotidase, has a very broad distribution (1.12 to 1.18 g/ml) and suggests that the kidney PM may exhibit a similar heterogeneity to that of the liver microsomal PM [28]. However, the brush border marker, alkaline phosphatase, has a more defined peak at density 1.175 (not illustrated). This difference between these two plasma membrane enzyme distributions may represent different properties of luminal and basolateral membranes. The most interesting region is the very light density of the front of the 5′-nucleotidase peak which is only found in the membrane of the Golgi apparatus [65], but may also represent a specialized region of the PM or endocytic vesicles.

Discussion

In these experiments we have separated three distinct populations of lysosomes from the rat kidney cortex in addition to numbers of very small acid hydrolase containing particles found in the microsomal fraction. This observed heterogeneity, also noted in previous studies [3, 5, 15], may represent either the known variations in lysosomal enzymes between cells in different parts of the nephron [19] or heterogeneity within single cell types. To address this problem, the following observations

provide evidence for the likely origins of the three major populations of lysosomes.

Large lysosomal granules in proximal tubule cells have been observed in extensive morphological studies [6, 12, 15]. Studies with microdissected nephron segments also demonstrate that the peak of lysosomal enzymes in the proximal convoluted tubule [2] parallels the lysosomal volume in this region [16]. Our data show that large granules from the ML fraction enriched with acid hydrolases sediment very rapidly (Fig. 1) and thus represent large lysosomes, which are only seen in the cells of the proximal tubule. Morphological examination of these lysosomes (Fig. 2E) shows large dense granules of 3 to 5 μ diameter consistent with proximal tubule lysosomes described by Straus [3]. Equilibrium banding of these lysosomes (Fig. 4) yields a homogeneous band at the unusually high median density of 1.235 g/ml, which is also consistent with the banding densities of lysosomes from the proximal tubule [11].

Filtered proteins are rapidly taken up mainly in the convoluted proximal tubule and somewhat less in the straight segment [17, 66, 67]. Following injection (i.v.) of ^{125}I lysozyme and ^{109}Cd-thionein, which are both avidly taken up by the proximal tubule cells [68, 69], 30 minutes before fractionating the cortical ML, we have shown that these two proteins are indeed rapidly incorporated into the large lysosomes.

While the large lysosomes fractionated in these studies appear to be derived essentially from the proximal tubule cells, the origins of the different small lysosome populations cannot be so definitively ascribed. Since lysosomes are present (in varying proportions) in virtually all the cells of the nephron, the cortical homogenate most likely contains populations of lysosomes from all the tubular segments. It is interesting to note that the lysosomes of the distal tubule cells, although few in number and/or small in size, are extremely rich in some acid hydrolases, since in relation to the relatively small volume the lysosomal enzyme activity is high [2].

Equilibrium banding yields two populations of small lysosomes at a density of 1.20 and 1.235 g/ml (Fig. 3). The distinct populations of less dense lysosomes is unusual because it apparently contains no cathepsin D or acid RNase. Because lysosomes of the distal tubule are relatively rich in acid phosphatase, NAG and acid β-galactosidase it is possible that at least a proportion of the small light lysosomes may originate from the distal tubule, particularly since the peak at density 1.20 coincides with a distinct peak of all these three enzymes. Although the distribution of ^{125}I and ^{109}Cd suggest that some endocytosed protein (lysozyme and Cd-thionein) is present in small lysosomes, this does not necessarily mean that these lysosomes must originate entirely from the proximal tubule. While little is known about the function of lysosomes in the distal tubule, the relatively high acid hydrolase activity suggests that some uptake of proteins may occur in this part of the nephron. Protein uptake by cells of more distal nephron segments (Henle's loop, distal tubule, collecting duct) has been reported, although quantitatively this represents a minor proportion of the reabsorbed proteins [6].

From the proportion of lysosomal enzymes and labelled proteins in the microsomal fraction we would conclude that, in agreement with other workers [8–10], this fraction contains very small lysosomes which are predominantly light lysosomes (density of 1.20 g/ml or less). However, our current data indicates that the microsomal fraction also contains lysosomal fragments or perhaps newly formed endosomes. Such membranous structures may originate from various regions of the nephron, as kallikrein which is an exclusive marker for the distal tubule [70] is predominantly found in microsomal vesicles (1.18 g/ml) [71].

The distribution pattern of renin (originating from glomerular epithelioid cells), which overlaps with the pattern of acid hydrolases in the broad band of small lysosomes also illustrates heterogeneity attributable to different and minor cell types. This renin distribution (Fig. 1) appears to be similar to that of 5′-nucleotidase and alkaline phosphatase. However, since only 3% of the total renin in the homogenate is recovered in the microsomal fraction, against 35% of the brush border enzyme alkaline phosphatase, we assume that renin is associated with renin granules and not brush border. Since the narrow peak of renin differs from the broad pattern of acid hydrolases, this suggests, in agreement with other reports [22, 24–27], that renin granules containing acid hydrolases [72] are distinct from small lysosomes. As stress situations, such as i.v. injections of labelled protein, are reported to increase renin release [73], the renin pattern from the isopycnic respin was considered atypical, and is therefore not illustrated.

Small dense lysosomes (1.235 g/ml) are very similar to protein droplets in having all the lysosomal enzymes assayed, containing endocytosed labelled protein and having the same boyant density. We also have evidence that cathepsin B, another lysosomal proteinase of the proximal tubule [74], is only present in lysosomal populations of high density (1.235 g/ml) in the kidney cortex [75]. These small lysosomes are very similar in properties, although not in size to the large lysosomes, and therefore may also originate from the proximal tubule cells. It is thus possible that the small dense lysosomes may represent structures intermediate between endocytic vesicles and large lysosomes, such as immature large lysosomes or newly formed phagosomes reported to be distinct from lysosomes [17].

Fractionation of lysosomes, by equilibrium banding, from isolated proximal tubules of perfused rabbit kidney suggest that only a single population, by density (at 1.216 g/ml), is present in the tubules of the rabbit kidney [76]. While these results support our findings that dense lysosomes are mainly of proximal tubule origin and also by inference that light lysosomes may therefore be of distal origin, they do not rule out the possibility that the apparently single population may contain subpopulations of proximal tubule lysosomes. The single population of lysosomes in the rabbit proximal tubules may alternatively reflect properties of lysosomes specific to that species or indeed specific to isolated tubules.

Preliminary experiments, showing that in metrizamide gradients cortical lysosomes band at two distinct densities (unpublished results) and observations that in Percoll gradients they also band at two different densities [77], suggest that this fundamental difference in density cannot be attributed to specific permeability differences of the different lysosomal populations to sucrose.

Time course studies with ^{3}H labelled Cd-thionein (Table 3) indicate extremely rapid breakdown which probably starts in the endosome compartment, as proteolytic activity in this compartment is reported [77] to occur in macrophages. The significant proportion of ^{3}H label in the supernatant is most

probably due to the contents of the endocytic compartment being released during homogenization, since the supernatant 10 minutes after injection contains undegraded ^{3}H labelled Cd-metallothionein as judged by fractionation with Sephadex G-75. The ^{3}H label in the 30 minute (and 1.5 hr) supernatant, fractionated by G-75, is associated only with low molecular-weight material indicative of very rapid breakdown of this protein (unpublished observations). The loss with time of ^{3}H label from microsomes and the corresponding increase of ^{3}H in the ML, and the relative increase in the large lysosomes strongly suggests transfer of some undegraded Cd-thionein from endocytic vesicles to small lysosomes and to large lysosomes. The breakdown of Cd-thionein in lysosomes thus appears to be slow in relation to the rapid breakdown in the endosome compartment. It is not clear if the two distinct rates of breakdown are typical for all proteins or if they are specific to just thionein. Thionein may be taken up both by fluid phase and receptor mediated endocytosis, and the two rates of breakdown reflect the two modes of uptake. To study these rates of breakdown we propose to investigate the comparative time course of uptake and catabolism of a number of labelled proteins in the rat kidney.

Our interpretations are based on the observations that the uptake and breakdown of perhaps most proteins (including thionein) occur in the proximal tubule cells. While catabolism probably occurs in this specialized portion of the nephron, our results, however, do not exclude the possibility that the different rates of turnover of Cd-thionein may be attributable to different regions of the tubule, thus reflecting the heterogeneity of the different cell types.

Acknowledgments

This work was supported by the Norwegian Council for the Sciences and the Humanities and in part by the British Council. We are grateful to Dr. D.W. Skagen for providing the computer programs used for presentation of results and to Dr. B.M. Mullock and Dr. R.H. Hinton for reviewing the manuscript. The antiserum anti-angiotensin I was kindly donated by Professor K. Poulsen, Institute for Biochemistry, The Royal Dental College, Copenhagen, Denmark.

Reprint requests to Dr. Knut-Jan Andersen, Medical Department A, University of Bergen, N-5016 Haukeland Sykehus, Bergen, Norway.

References

1. TISCHER CC: Anatomy of the kidney, in *The Kidney* (2nd ed.), edited by BRENNER BM, RECTOR FC. Philadelphia-London-Toronto, WB Saunders & Co., 1981, pp. 3–75
2. LE HIR M, DUBACH UC, SCHMIDT U: Quantitative distribution of lysosomal hydrolases in the rat nephron. *Histochemistry* 63: 245–251, 1979
3. STRAUS W: Concentration of acid phosphatase, ribonuclease, desoxyribonuclease, β-glucuronidase, and cathepsin in "droplets" isolated from the kidney cells of normal rats. *J Biophys Biochem Cytol* 2:513–521, 1956
4. STRAUS W: Segregation of an intravenously injected protein by "droplets" of the cells of rat kidneys. *J Biophys Biochem Cytol* 3:1037–1040, 1957
5. STRAUS W: Isolation and biochemical properties of droplets from the cells of rat kidney. *J Biol Chem* 207:745–755, 1954
6. STRAUS W: Occurrence of phagosomes and phago-lysosomes in different segments of the nephron in relations to the re-absorption, transport, digestion, and extrusion of intravenously injected horseradish peroxidase. *J Cell Biol* 21:295–308, 1964
7. WATTIAUX-DECONINCK S, RUTGEERTS MJ, WATTIAUX R: Lysosomes in rat kidney tissue. *Biochim Biophys Acta* 105: 446–459, 1965
8. SHIBKO S, TAPPEL AL: Rat kidney lysosomes: Isolation and properties. *Biochem J* 95:731–741, 1965
9. PRICE RG, DANCE N: The cellular distribution of some rat kidney glycosidases. *Biochem J* 105:877–883, 1967
10. GOLDSTONE A, KOENING H: Biosynthesis of lysosomal glycoproteins in rat kidney. *Life Sci* 11:511–532, 1972
11. MANUSBACH AB: Isolation of kidney lysosomes, in *Methods in Enzymology: Biomembranes*, part A (Vol. 31) edited by FLEISCHER S, PACKER L. New York, Academic Press, 1974, pp. 330–339
12. MANUSBACH AB: Isolation and purification of acid phosphatase-containing autofluorescent granules from homogenates of rat kidney cortex. *J Ultrastruct Res* 16:13–34, 1966
13. IRINTSCHEFF A, DAVIDOFF M: Ueber die verteilung einiger hydrolasen in der rattenniere. *Histochemistry* 71:463–480, 1981
14. ROSS BD, GUDER WG: Heterogeneity and compartmentation in the kidney, in *Metabolic Compartmentation*, edited by SEIS H. London, Academic Press, 1982, pp. 363–409
15. MAUNSBACH AB: Observations on the ultrastructure and acid phosphatase activity of the cytoplasmic bodies in rat kidney proximal tubule cells. With comment on their classification. *J Ultrastruct Res* 16:197–238, 1966
16. PFALLER W, RITTINGER M: Quantitative morphology of the rat kidney. *Int J Biochem* 12:17–22, 1980
17. STRAUS W: Cytochemical observations on the relationship between lysosomes and phagosomes in kidney and liver by combined staining for acid phosphatase and intravenously injected horseradish peroxidase. *J Cell Biol* 20:497–507, 1964
18. KRETCHMER N, BERNSTEIN J: The dynamic morphology of the nephron: Morphogenesis of the protein droplets. *Kidney Int* 5: 96–105, 1976
19. DAVIES M: The heterogeneity of lysosomes, in *Lysosomes in Biology and Pathology*, (Vol 4) edited by DINGLE JT, DEAN RT. Amsterdam, North Holland Publ. Co., 1975, pp. 305–348
20. GOLDSTONE A, KOENING H, NAYYAR R, HUGHES C, LU CY: Isolation and characterization of a rough microsomal fraction from rat kidney that is enriched in lysosomal enzymes. *Biochem J* 132:259–266, 1973
21. BARAJAS L, MULLER J: Structure of the juxtaglomerular apparatus, in *The Renin-Angiotensin System*, edited by JOHNSON JA, ANDERSON RR. New York–London, Plenum Press, 1980, pp. 85–131
22. MORIMOTO S, YAMAMOTO K, UEDA J: Isolation of renin granules from the dog kidney cortex. *J Appl Physiol* 33:306–311, 1972
23. BEAUFAY H: Methods for the isolation of lysosomes, in *Lysosomes. A Laboratory Handbook*, edited by DINGLE JT. Amsterdam–London, North–Holland Publ. Co., 1972, pp. 1–45
24. SAGNELLA GA, PEART WS: Studies on the isolation and properties of renin granules from the rat kidney cortex. *Biochem J* 182:301–309, 1979
25. SAGNELLA G, PRICE R, PEART W: Subcellular distribution and storage form of rat renal renin. *Hypertension* 2:595–603, 1980
26. MANISTÖ PT, POISNER AM: Isolation of renin granules from rat kidney cortex by isotonic or hyperosmotic metrizamide–sucrose gradients. *Prep Biochem* 10:297–316, 1980
27. MANNISTÖ PT, POISNER AM: Further studies on properties of renin granules isolated from rat kidney cortex. *Acta Physiol Scand* 112:365–371, 1981
28. NORRIS KA, DOBROTA M, ISSA FS, HINTON RH, REID E: Heterogeneous distribution of enzymes among plasma-membrane fragments sedimenting with the microsomal fraction of rat liver. *Biochem J* 142:667–671, 1974
29. BRADFORD M: A rapid and sensitive method for the quantitation of microgram quantities of protien utilizing the principle of protein–dye binding. *Anal Biochem* 72:248–254, 1976
30. SCHUEL H, SCHUEL R: Automated determination of protein in the presence of sucrose. *Anal Biochem* 20:86–93, 1967
31. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with Folin–phenol reagent. *J Biol Chem* 193:265–275, 1951
32. HINTON RH, NORRIS KA: Effect of sucrose on continuous-flow analyzers. *Anal Biochem* 348:247–258, 1972
33. FLECK A, BEGG D: The estimation of ribonucleic acid using

ultraviolet absorption measurements. *Biochim Biophys Acta* 108:333–339, 1954
34. NOBBS BT, WALKER AW, DAVIES TJ: A simplified method for the estimation of urinary total hydroxyproline. *Clin Chim Acta* 64:219–221, 1975
35. MORRE DJ, CLINE GB, COLEMAN R, EVANS WH, GLAUMANN H, HEADON DR, REID E, SIEBERT G, WIDNELL CC: Markers for membraneous cell components. *Eur J Cell Biol* 20:195–199, 1979
36. PENNINGTON RJ: Biochemistry of dystrophic muscle. Mitochondrial succinate–tetrazolium reductase and adenosine triphosphate. *Biochem J* 80:649–654, 1961
37. KRAJL M: A rapid microfluorimetric determination of monoamine oxidase. *Biochem Pharmacol* 14:1683–1685, 1965
38. WEISSBACH H, SMITH TE, DALY JW, WITHOP B, UDENFRIEND S: A rapid spectrophotometric assay of monomine oxidase based on the rate of disappearance of kynuramine. *J Biol Chem* 235: 1160–1163, 1960
39. LEIGHTON F, POOLE B, BEAUFAY H, BAUDHUIN P, COFFEY JW, FOWLER S, DE DUVE C: The large–scale separation of peroxisomes, mitochondria and lysosomes from the livers of rats injected with triton WR-1339. Improved isolation procedures, automated analysis, biochemical and morphological properties of fractions. *J Cell Biol* 37:482–513, 1968
40. MIRCHEFF AK, WRIGHT EM: Analytical isolation of plasma membranes of intestinal epithelial cells. Identification of Na, K-ATPase rich membranes and the distribution of enzyme activities. *J Membrane Biol* 28:309–333, 1976
41. HÜBSCHER G, WEST GR: Specific assays of some phosphatases in subcellular fractions of small intestinal mucosa. *Nature (London)* 205:799–800, 1963
42. SCHWARTZ A, NAGANO K, NAKAO M, LINDENMAYER GE, ALLEN JC, MATSUI H: The sodium– and potassium–activated adenosinetriphosphate system. *Meth Pharmacol* 1:361–388, 1971
43. BARRETT AJ: Lysosomal acid proteinase of rabbit liver. *Biochem J* 104:601–608, 1967
44. HULTBERG B, ÖCKERMAN PA: Artificial substrates in the assay of acid glycosidases. *Clin Chim Acta* 39:49–58, 1972
45. DOBROTA M, BURGE MLE, HINTON RH: Apparent heterogeneity of hepatic lysosomes due to membrane–bound acid phosphatase. *Eur J Cell Biol* 19:139–144, 1979
46. EL-ASER AA, REID E: Rat liver 5′-nucleotidase. *Histochem J* 1:417–437, 1969
47. DOBROTA M, HINTON RH: Large–scale preparation of highly purified lysosomes from normal rat liver. *Anal Biochem* 102: 97–102, 1980
48. POULSEN K, JORGENSEN J: An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody. *J Clin Endocr Metab* 39:816–825, 1974
49. ANDERSEN K-J, DOBROTA M, HAGA HJ: The effect of sucrose on assaying enzymes and protein in the subcellular fractions of the rat kidney cortex. *J Biochem Biophys Meth* 1:309–311, 1979
50. FRAKER PJ, SPECK JC: Protein and cell membrane iodinations with sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril. *Biochem Biophys Res Com* 80:849–857, 1978
51. KAGI JHR, NORDBERG M: Metallothionein and other low molecular weight metal–binding proteins. *Experientia Supplementum* 34: 41–124, 1979
52. DOBROTA M, BONNER FW, CARTER BA: Intracellular redistribution of cadmium–thionein following its uptake into the renal cortex, in *Nephrotoxicity: Assessment and Pathogenesis,* edited by BACH PH, BONNER FW, BRIDGES JW, LOCK EA. Chichester, Wiley, 1981, pp. 320–324
53. DOBROTA M, HAGA HJ, ANDERSEN K-J: The effect of glomerulonephritis on the tubular uptake of cadmium-thionein by the rat kidney, in *Renal Heterogeneity and Target Cell Toxicity,* edited by BACH PH, LOCK EA. Chichester, Wiley, 1985, pp. 241–244
54. TANG YS, DAVIS A-M, KITCHER JP: N-Succinylpropionate characterization and optimum conditions for use as a tritium labelling reagent for proteins. *J Lab Comps Radiopharm* 20:277–284, 1983
55. ANDERSEN K-J, HAGA H, DOBROTA M: Heterogeneity of rat kidney cortex lysosomes fractioned by gradient centrifugation in zonal rotors. *Biochem Soc Trans* 8:597–598, 1980
56. FURTH A, ROBINSON D: Specificity and multiple forms of β-galactosidase in the rat. *Biochem J* 97:59–66, 1965
57. STRAUS W: Changes in "droplet" fractions from rat kidney cells after intraperitoneal injection of egg white. *J Biophys Biochem Cytol* 3:933–947, 1957
58. MASTERS CJ, HOLMES R: Peroxisomes: New aspects of cell physiology and biochemistry. *Physiol Rev* 57:816–882, 1977
59. MEIER PJ, SPYCHER MA: Isolation of a subfraction of rough endoplasmic reticulum closely associated with mitochondria. Evidence for its role in cytochrome P450 synthesis. *Exp Cell Res* 111:479–483, 1978
60. SCHNAITMAN C, GREENWALT JW: Enzymatic properties of the inner and outer membranes of rat liver mitochondria. *J Cell Biol* 38:158–175, 1968
61. KEMPSON SA, PRICE RG: A comparison of some enzyme activities associated with plasma membrane preparations from the cortex and other medullar of the rat kidney. *Int J Biochem* 10:67–73, 1979
62. SPIRO RG: Basement membranes and collagens, in *Glycoproteins* (2nd ed), edited by GOTTSCHALK A. Amsterdam, Elsevier Publishing Company, 1972, pp. 964–999
63. DALLNER G: Studies on the structural and enzymic organization of the membrane elements of liver microsomes. *Acta Path Microbiol Scand* (suppl) 166:1–94, 1963
64. BEAUFAY H, JAQUES P, BAUDHUIN P, SELLINGER OZ, BERTHET J, DE DUVE C: Tissue fractionation studies. 18. Resolution of mitochondrial fractions from rat livers into three distinct populations of cytoplasmic particles by means of density equilibrium in various gradients. *Biochem J* 92:184–205, 1964
65. WIBO M, THINES-SEMPOUX D, AMAR-COSTESEC A, BEAUFAY H, GODELAINE D: Analytical study of microsomes and isolated subcellular membranes from rat liver. VIII. Subfractionation of preparations enriched with plasma membranes, outer mitochondrial membranes or Golgi complex membranes. *J Cell Biol* 89:456–474, 1981
66. MAUNSBACH AB: Absorption of ^{125}I labelled homologous albumin by rat kidney proximal tubule cells. *J Ultrastruct Res* 15:197–241, 1966
67. MAACK T, PARK CH, CAMARGO MJF: Renal filtration, transport, and metabolism of proteins, in *The Kidney: Physiology and Pathophysiology,* edited by SELDIN DW, GIEBISCH G. New York, Raven Press, 1985, pp. 1773–1803
68. MAACK T, MACKENSIE DDS, KINTER WB: Intracellular pathways of renal absorption of lysozyme. *Am J Physiol* 221:1609–1616, 1971
69. SQUIBB KS, RIDLINGTON JW, CARMAICHAEL NG, FOWLER BA: Early cellular effects of circulating cadmium–thionein on kidney proximal tubules. *Env Hlth Perspect* 28:287–296, 1979
70. Omata K, Carretero OA, Scicli G, Jackson BA: Localization of active and inactive kallikrein (kininogenase activity) in the microdissected rabbit nephron. *Kidney Int* 22:602–607, 1982
71. ANDERSEN K-J: The subcellular distribution of rat kidney cortex angiotensin–converting enzyme in relation to renin and kallikrein, in *Recent Progress in Kinins. Agents and Actions* (suppl vol 9), edited by FRITZ H, DIETZE G, FRIEDLER F, HABERLAND GL. Basel, Birkhauser, 1982, pp. 559–563
72. TAUGNER R, WHALLEY A, ANGERMULER S, BUHRLE CP, HACKENTHAL E: Are the renin–containing granules of juxtaglomerular epithelioid cells modified lysosomes. *Cell Tissue Res* 239:575–587, 1985
73. IVERSEN BM, ANDERSEN K-J: The effect of sampling conditions on rat plasma renin. *Comp Biochem Physiol* 74A:331–332, 1983
74. KUGLER P: Demonstration of cathepsin B in the rat kidney using fluorescence histochemistry. *Histochemistry* 82:299–300, 1985
75. ANDERSEN K-J, DOBROTA M: The lysosomal distribution of cathepsin B in the rat kidney cortex. *Renal Physiol* (in press)
76. HJELLE JT, MORIN JP, TROUET A: Analytical cell fractionation of isolated rabbit renal proximal tubules. *Kidney Int* 20:71–77, 1981
77. PILLAI S, ZULL JE: ATP activation of protein degradation by extracts of crude and purified lysosomal preparations. *Biochim Biophys Acta* 843:92–100, 1985
78. DIMENT S, STAHL P: Macrophage endosomes contain proteases which degrade endocytosed protein ligands. *J Biol Chem* 260: 15311–15317, 1985